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Subject-matter of the invention are a system for the release and isolation of nucleic acids and a method for use system.

Proof procedures, which are based on the identification of nucleic acids in a sample, were considered in recent time amplified with interest. This lies among other things in the attainable high specificity of the proof. Herein nucleic acid oh way the antigen proofs is superior in principle. While antigens are however often present in a sample already relatively accessible, nucleic acids, in particular with proofs by organisms, must be made accessible usually in several steps. Beyond that nucleic acids are usually in very small concentrations present. In particular with the isolation of nucleic acids from zellhaltigen samples complex Aufreinigungsverfahren is well-known.

At present the sample enrichment and sample preparation systems for nucleic acids, offered on the market, do not make a purposeful enrichment possible of cells with magnetic particles. The sensitivity with these methods is often not sufficiently hoc at present available automatically working sample preparation systems needs organic solvents (phenol and/or chloroform alcohol mixtures) for the production of nucleic acid.

The methods using an immobilization of nucleic acids, used at present, use essentially two principles for the isolation nucleus sows. In a first possibility nucleus-acid liquid samples are sucked through a solid phase matrix, whereby nucleic acids are held in the solid phase matrix. This presupposes a previous Lyseschritt, which was accomplished in a separate vessel. Subsequently, nucleic acids are loosened by sucking a Elutionsflüssigkeit through from the solid phase matrix. The nucleus-acid Elutionslösung is sucked off into a vessel for further processing. It turned out however that the apparatuses regarding for the lead-through of a later amplification reaction, z, used at present. B. PCR, necessary purity is not sufficient.

With a second principle nucleic acids are precipitated and separated by means of a centrifuge. With this method however a so-called batch operating is inevitable. With such methods for example a zellhaltige solution in a first reaction container is treated with lysierenden agents. Subsequently, the reaction mixture from the vessel is umpipettiert in a Zentrifugationsröhrchen. This tube has an inset, at which set free nucleic acids can adsorb, while the remaining fluid can flow during the Zentrifugation into the lower range of the tube. To the washing of absorbed nucleic acids the inset in or several times is treated with a washing liquid. For this the inset must be transferred in a further Zentrifugationsröhrchen, so that remainders of the sample liquid do not arrive again back into the inset. In the last step the inset is used into a further new vessel. Nucleic acids would be transferred of a Elutionslösung by Zentrifugation by the inset into a further vessel inside into a subsequent treatmentable solution. This method is however on the one hand afflicted with a high contamination risk and on the other hand is necessary a multiplicity of changes of the reaction containers.

Task of the present invention was it to make a system available with which the disadvantages of the state of the art are at least partly eliminated vollstän or. In particular nucleic acids at a solid phase matrix can be desorbed absorbi and with this system, without for these steps a centrifuge would be necessary.

A core of the invention is the use more simply, usually in automatic analyzers of occurring modules for the operation of the system.

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Subject-matter of the invention is containing a method for the release and isolation or to the detection of nucleic acids from biological Kompartimenten of a sample, the steps:

- Inkubation of the sample in a sample working on container as well as magnetic particles, which can bind the biological Kompartimente, under vibrating the sample working on container,
- Positioning a magnet in the proximity of the sample working on container, so that the magnetic particle to the Gefässwand are held,
- Removing the resulting fluid from the sample working on container,
- Resuspension of the magnetic particle in a second fluid through

a) Remove the magnet from the proximity of the sample working on container, so that the magnetic particle no more are not held by the magnet to the wall and simultaneous
b) Vibrate the sample working on container,

- Explanation of the biological Kompartimente under heating up,
- Cooling of the explanation mixture under conditions, which make an immobilization or a hybridizing for that possible to insulating or nucleic acid which can be proven.

Likewise subject-matter of the invention is a system for the release and isolation of nucleic acids from a suspension of biological Kompartimenten with magnetic particles.

Nucleic acids in the sense of the present invention are nucleic acids, which are present in biological Kompartimenten.

Under biological Kompartimenten become in particular cells, z. B. viralen or bacterial origin understood. Particularly preferentially the cells in substantial isolated condition are present. In principle also more-cellular Kompartimente in the sense of the invention can be worked on. This Kompartimente with its nucleic acids is present at the beginning of the method according to invention in a sample. This sample a suspension D biological Kompartimente is preferential in a fluid. Such samples can be for example received from body fluids, z. Blood, saliva or urine.

By release of nucleic acids in the sense of the invention the withdrawal of nucleic acids from the biological Kompartimenten is understood. This withdrawal can happen in arbitrary way. Preferred the withdrawal takes place by destruction of the wall defining the biological Kompartimente against the fluid. This can be for example achieved by treatment of the Kompartimente with cell wall-destructive compositions, z. B. Proteinase K.

By the isolation of nucleic acids the separation of the nucleus SAE urn is understood about other components of the sample. Such other components are for example the walls of the biological Kompartimente, their dismantling products, further contents materials of the biological Kompartimente as well as contents materials of the fluid, which surrounds the biological Kompartimente in the sample. To it for example proteins, inhibitors for enzymes, in particular nucleic acid-diminishing enzymes, belong like DNase. In this sense isolation can be understood also as a kind purification of nucleic acids. This isolation can be both specific and nonspecific regarding further nucleic acids contained in the sample.

By a detection of nucleic acids a method is understood according to invention, with which the presence or quantity of nucleic acids is determined. These methods can be made both quantitatively and qualitatively. For the lead-through of quantitative proofs a comparison attempt with a Pr is usually accomplished, which contains a well-known quantity of nucleic acids which can be proven. The proof can be both sequence specific and sequence nonspecific. In order to make the proofs specific, one uses usually so-called probes, which are characterized by the fact that they exhibit a Nukleobasensequenz, which is more or less characteristic of nucleic acids in the sample. If a specific proof is wished by nucleic acids, a sonde is used, which contains a cousin sequence, which complementary can be to the cousin sequence of nucleic acid, not however to other nucleic acids in the sample, which can be proven, is sondes molecules, which contain one directly or of indirect provable group. Directly provable groups are for example radioactive (< 3> < 2> P) colored or fluorescent groups or metal atoms. Indirect provable groups are for example immunological or enzymatically effective connections, like antibody, antigens Haptene, enzymes or enzymatically active partial enzymes. These are detected in a following reaction or reaction sequence. Haptene, z are particularly preferential. Digoxygenin or Biotin.Solche haptenmarkierten sondes can be easily proven in a following reaction with a labeled antibody against the Hapten.

Inkubiert in a first step the sample in a sample working on container as well as magnetic particles (Beads), which the biological Kompartimente bind can, under vibrating the sample working on container. By magnetic particles particles are understood, which by a magnet into a certain direction to be transported to be able. To it for example ferromagnetic or super+paramagnetic materials belong. In the sense of the invention ferromagnetic materials are particularly preferential. Particles are solid materials with a small diameter. In the sense of the invention particularly particles are suitable, which have an average grain size of more as 2.8 mu m, however less than 200 mu m. Particularly preferentially wise it an average grain size between 10 15 mu m up. Preferentially the grain size distribution is homogeneous. These particles are so modified at their surface that they can bind the biological Kompartimente. For this suitable magnetic particle are the well-known and available Latexmagnetpartikel to which z. B. Antibodies to be bound can. For the connection of the biological Kompartimente to the magnetic particle in particular antibodies are used, which are directed against surface antigens of the biological Kompartimente. Such magnetic particle are likewise commercially available.

The sample working on container is preferentially in a construction unit 10 of the system, which is suitable for the solid admission of the sample working on container. The construction unit can take up also several vessels. Particularly preferentially this construction unit exists in a plate, in which so many holes are, how vessels are to be taken up. The holes are watched out in their geometry for the vessels. The fixing of the vessels in the construction unit is in such a way arranged bevorzu that the vessels can be taken from the treatment of samples after lead-through in a simple manner again. Preferred at the construction unit a tube fastens, the underpressure of a sucking off unit, z. B. a vaccum pump, up to the hole in D construction unit 10 and thus, with put on sample working on container, until at its discharge opening leads. In the case of the creation of an underpressure therefore fluid becomes and/or. Air from the sample working on container by the tube to the pump promoted. Suitable valves are preferentially so controlled that the underpressure rests against the sample working on container only if a promoting is to take place.

The Inkubation of the samples with the magnetic particles can be arranged in arbitrary way. It is necessary that both di Probe and the magnetic particles are brought into the sample working on container. Both the kind of bringing in and their sequence is in principle without greater importance for the method according to invention. Preferentially however the magnetic particles in form of a suspension with a well-known content of magnetic particles are pipetted into the sample working on container. Either afterwards or before the sample is in-pipetted into the sample working on container.

The Inkubation is made under suitable conditions until a sufficient quantity of biological Kompartimen is bound to the magnetic particle. Here as a rule one period between 1 min and 10 min will concern. The sample working on container is here preferential in suitable way, z. B. by means of a cover and/or a valve sealed.

A substantial feature of the invention is that the mixture in the sample working on container is vibrated during the Inkubati. It can concern here an interval vibrating. Vibrating can become however also during the entire incubation period or only parts of it durchgefüh. Vibrating serves to reach a sufficient mixture of the biological Kompartimente and the magnetic particle in the liquid in particular the suspension and/or. Resuspension of the Beads and the acceleration of diffusion. Thereby the time of the Inkubation necessary for the connection of the biological Kompartimente to the magnetic particle is reduced.

Vibrating is reached by movement of the sample working on container, preferentially in horizontal direction. Particularly preferred a unit 10, which photographs (holes) with one or more sample containers contains, is moved, so that all sample containers in it are along-vibrated. In the sense of the invention the use of a unit 30, which does not accomplish the movement of the sample working on containers (A) manual, is preferential. This unit can be each mechanical equipment suitable in principle for mixing fluids in a vessel. A preferential example of such a unit is described in the following.

A stepping motor with a Excenter and a balance mass the complete DNA module (unit 10), put on over vibration dampers on this frameworks, drives solid amplitude and variable frequency into a circular eccentric course from a solid framework 1. The preferential amplitude is A @ 1.5 mm, the preferential frequency 1 @ f @ 50 cycles per second. The mixing and/or. Resuspensionsdauer amounts to depending upon physical properties of the sample material between 5 and 30 S. By replacement of the Excenters it is possible in addition, in few minutes for the service to vary the amplitude manual.

The combination of the system according to invention with a pipetting automat is not as such being obvious, since for this planning of a defined positioning of the sample containers is necessary during the pipetting steps before and. Vibrating the vessels leads otherwise to the fact that after each the vessels are vibration procedure at another position. If the deflection of the path of the vessels would lead to the fact that the pipetting automat pipettes one into the vessel liquid which can be pipetted beside the vessel, would be practically badly possible normal accomplishing of an automated method. Therefore for it it is provided that the vessel is after vibrating in a defined so-called Home position, in which a pipetting or other events to take place to be able.

The inset of a stepping motor is favourable opposite the inset of a DC engine for the defined Home position and the inset with the pipetting full automat. The Home position is detected with a light barrier.

Regarding the constructional execution exist still the following non invasiven alternative possibilities, however all this the construction (1. and 2.) more complex or in the mixing steps (3.) longer last:

1. A combination of, two or three linear drives in the level and/or. in the area (x, Y, Z-axis) to the production of z. B. Lissajous figures.
2. Tumbles, swivelling or knocking the DNA module.
3. Magnet agitator.

The sample working on container (A) can exhibit any form in principle. Such sample working on containers know z. B. the indentation of a micro titer plate, z. B. in 96 the waving format, its. Preferred it concerns however an essentially hollow-cylindrical vessel, which prefers an upper inlet port and, particularly, a lower discharge opening contains. Such a sample working on container can be used for the contamination-reduced treatment of nucleus-acid samples. These vessels consist preferentially of plastic, z. B. Polypropylene.

Following the Inkubation and connection of the Kompartimente to the magnetic particle the biological Kompartimente is removed for surrounding fluid of the sample from that it. For this it proved as appropriate to collect the magnetic particle with the biological Kompartimenten bound to it by positioning of a magnet in the proximity of the sample working on container. Thereby prefers the magnetic particle with the biological Kompartimenten held to the Gefässwand. In the sense of the invention, when particularly prefers, for the positioning of the magnets a unit (40) with one or more permanent magnets or electromagnets to the sample working on container is driven near. The resulting distance of the magnet of the sample working on container depends strongly on the value of the magnetic field attainable by the magnet and the value and magnetizability of the magnetic particle. In addition the kind of the later following working on steps (z has. B. mechanical load of the magnets) an influence on the magnetizing force which can be used. If it concerns a permanent magnet, this is brought from a position, which is not sufficient for a separation of the magnetic particle during the Inkubationsschrittes, into the proximity of the vessel, so that the magnetic particle to the Gefässwand are held. For the case of the use electromagnets this is switched on and in the switched on condition left until a treatment of the held biological Kompartimente is final.

By the positioning of a magnet in the proximity of the vessel also the case is to be understood that the vessel is brought into the proximity of the magnet. Finally it depends thus only on the relative motion of the magnet to the vessel.

The unit (40) exhibits prefers a magnet, which on a pre-determined course, z. B. over strips or, z prefers by movement of the magnet on a circular path. B. around beside the sample container lying an axle, on the sample working on container movable is. In addition for this counts an engine, which can realize both the movement of the magnet on the sample working on container too and its moving away. Preferred the unit (40) exhibits a toothed belt, which changes the rotary motion of the DC engine on a side of the DNA of module with in each case four shafts to the admission of 4 magnets each and toothed wheel at the faces into the circulation of the magnets. The two final positions are detected with in each case a light barrier. On the opposite side of the DNA module is exactly the same arrangement, to move so that the magnets of each side synchronously opposite one on the other. In this case twice as many magnets are used such as vessels. In the case of example is the radius of the circular path approx. 8 mm and the furthest spacing of the magnet of the sample container approx. 12 mm.

In an alternative arrangement for n vessels n+1 magnet are used. Here the same magnet between two neighbouring Tubes are led, one save thus n-1 [=2n (n+1)] Magnets. In the position ?ON? has the magnet maximum effect on the Magnetbeads. In the position ?OFF? is so far from tube the magnet that he does not have effect on the Magnetbeads. The driving time (t) between the end positions ?ON? and/or. ?OFF? amounts to prefers less than 1.5 S.

A further alternative is those relatively fixed positioning between magnet and vessel, but the movement of a shielding mu - metal between magnet and vessel.

The magnet exhibits a mass preferentially between 0,5 and 5 g, particularly preferentially between 1 and 4 g, in the particular case 2.3 G. The outside dimensions amount to 10 mm x 10 mm x 3 mm. As suitable material for a permanent magnet rare soils (z have themselves. B. NeFeBr, VACODYM 370 HR) with an optimal BH maximum with smallest dimensions proved. To that extent it is favourable to dimension the gradients of the magnetic field particularly pronouncedly. For this reason also the positioning of the magnet is to come as near as possible with the vessel. It is preferential selecting if possible sample working on containers which cause as small a damping of the magnetic field as possible, z. B. from polypropylenes.

Under the Gefässwand of the sample working on container for the separation of the Beads the inner wall or a part of it, which is present under the liquid surface of the sample, is usually used. Preferred it concerns a side wall of the vessel.

Subsequently, the biological Kompartimente surrounding fluid is removed from the sample working on container. This happens under conditions, with which the magnetic particle at the Gefässwand stay. The kind of the distance depends on the kind of the sample working on container. It knows z. B. are abpipettiert. In a preferential embodiment however, with which the sample working on container exhibits a lower discharge opening, the fluid is simply sucked off by these. This kind of the distance keeps the mechanical load of the magnetic particle small and avoids thus the separation of the magnetic particle of the Gefässwand.

A particularly important step is the Resuspension of the magnetic particle held back at the Gefässwand in an admitted second fluid. For this the magnet is removed from the proximity of the vessel, so that the magnetic particle no more are not held by the magnet to the Gefässwand. As described above, it is also possible to remove the vessel from the proximity of the magnet. According to the present invention did not prove the simple removing of the magnet as for a sufficient Resuspension, if not the vessel prefers supplementing, simultaneous is vibrated. This vibrating is accomplished again by the unit 30. It causes an even distribution of the magnetic particle in second this second fluid can before removing the magnet, however also only after removing the magnet into the sample working on container be filled, z. B. by a pipetting.

The method according to invention can be used even for the further Aufreinigung by biological Kompartimenten., for this a suspension of the magnetic particle, which the biological Kompartimente contains bound, in a sample working on container in relation for a magnet positioned that the magnetic particle with the biological Kompartimenten to the Gefässwand are held, afterwards the fluid, which contained the biological Kompartimente, from the vessel is in such a way removed and afterwards the magnetic particle in a second fluid, here a washing liquid, by removing the magnet from the proximity of the vessel, so that the magnetic particle no more are not held by the magnet to the Gefässwand, and simultaneous vibrating of the vessel resuspendiert. This washing process can be repeated at will, until one sufficient purity of the biological Kompartimente is reached.

As the further step of the method according to invention afterwards the explanation (Lyse) is the biological Kompartimente intended. Methods for the explanation of biological Kompartimente are just as well-known the person skilled in the art, as the specific conditions for certain kinds of Kompartimenten, z. B. Cells. For example for the explanation by bacteria the biological Kompartimente with a mixture is shifted by Proteinase K and inkubiert for a certain time, for breaking open and/or. the partial or complete digest the cell walls under release of nucleic acids contained in the biological Kompartimenten one inkubiert. Preferentially at temperatures over ambient temperature, particularly preferentially between 70 and 95 DEG C one works. The mixture, which is produced by the explanation of the cells, is called in the following also explanation mixture. The Inkubation is preferably accomplished over a time from 5 to 20, particularly preferentially between 10 and 15 minutes.

In particular, if the explanation of the cells took place at ambient temperature or slightly increased temperature, it is preferential heating the explanation mixture up afterwards on higher temperatures for example on 70 DEG C, or, with potentially infectious samples, on 95 DEG C. Here gewünschtenfalls also the Lysereagenz can be disturbed, inactivated, should it with further steps.

Heating and/or. Cooling of the fluid in the sample containers is made according to invention by a unit 20. This unit, which consists in principle of construction units usual for thermostats, is preferably partly into the unit 10, in which the sample working on containers can be positioned, integrated. It contains in particular a block made of metal, which has heat conducting properties. This is co-ordinated and preferentially on a liquid medium is thermostatisiert with the physical form of the sample working on containers. Depending upon reaction step which can be accomplished in the sample working on container the temperature of this block is increased and/or. degraded. As liquid medium well-known agents can serve. The medium preferentially over flexible tubes of a heating and/or. Cooling by means of a circulation pump into the block transports. The use of flexible hoses does not make possible also the attachment of the stationary components, like the heating, the cooling and the circulation pump on during the method according to invention 10 frameworks of the apparatus along-moved with the unit. This is made possible in particular by the fact that the deflections are only relatively small during the Schüttelbewegungen.

Subsequently, the explanation mixture is cooled down, under conditions, those are dependent of the purpose of the method according to invention. If an isolation of nucleic acids at a solid phase is to take place, conditions are stopped, with which to this solid phase can bind nucleic acid. A suitable method for the connection of nucleic acids is the Inkubation of set free nucleic acids with glass surfaces under presence of chaotroper salts. Such a method is for example described in EP-A-0 389,063. Here nucleic acids are bound in nonspecific way to the glass surface, while other components of the biological Kompartimente as well as the explanation reagents are not bound or only insignificantly to the glass surface. Preferred afterwards the fluid, which contains the remaining components, is taken out of the sample working on container, z. B. sucked off, while the glass surface with nucleic acids bound to it can remain in the sample working on container. To a preferential embodiment a solid phase is introduced into form of a glass fiber fleece into the sample working on container and inkubiert with the mixture. Thereby nucleic acids at the glass fiber immobilized will be able and in a simple manner with the glass fiber fleece out of the sample working on container to be taken.

If nucleic acids are to be proven after their release, these are hybridized with a sonde. With this sonde it concerns, as described above, a molecule, which exhibits a cousin sequence complementary to nucleic acid or a part of, it which can be proven. In a preferential case it concerns a Oligonukleotid, which with a provable group labeled is. The cooling down of the reaction mixture therefore takes place under conditions at which a hybridizing of nucleic acid with the nucleic acid probe, which can be proven, takes place. These temperatures are well-known a person skilled in the art. In another embodiment than methods to the proof of nucleic acids a hybridizing between nucleic acid which can be proven and a solid-phasebound nucleic acid probe takes place. Here the sonde can be used to any solid phase, as long as it is separable by the remaining reaction mixture only, z. B. Micro titer disk cavities or the inner wall of the sample working on container. Methods for the immobilization of nucleic acid probes, in particular the so-called catch probes, are well-known the person skilled in the art, z. B. from EP-A-0 523,557.

Generally a separation becomes the too insulating and/or to the cooling down of the mixture. nucleic acids of that, which can be proven, it surrounding fluid, which if necessary. still remainders of the explanation mixture and possibly. contains

of the reagents used for the connection of nucleic acids to a solid phase, attach. For this, depending upon kind of the used solid phase, a filtration or a distance of the solid phase from the sample working on container or Abpipettieren of the fluid from the sample working on container can be made.

Bound nucleic acids stand afterwards either for the abolition of their connection to the solid phase or their direct proof in usual, for the person skilled in the art admitted methods to the proof of nucleic acid sequences or a marker for order.

The method according to invention uses therefore a combination of working on steps, which use a unit 10 for the admission one or several sample working on containers, a unit 20 for the Thermostatisierung of the sample working on containers and in it contained fluids, a unit 30 for vibrating the sample working on containers and a unit 40 for the magnetic separation of the magnetic particle to a wall of each sample working on container. Surprisingly these process steps and units in only one reaction block can be implemented. As reaction block hereby an apparatus is understood, which partly or completely contains the units 10, 20, 30 and 40 in coupling co-ordinated one on the other. In way according to invention succeeds in a simple manner an event, which presupposed so far a multiplicity of manual work procedures, letting run off in only one apparatus. In particular it proved that reaction block according to invention the particularly effective are. Methods for release and isolation of nucleic acids can be accomplished with them quicker as so far. It is beyond that possible not to remove during the steps mentioned nucleic acid from the vessel. This represents both regarding the expenditure of time, and on the avoidance of contaminations a substantial progress opposite the state of the art. Usually so far coolings down were accomplished by suspensions by manual removal of a sample working on container from the apparatus and dipping of the vessel into a cooling bath. Such a procedure did not prove as for the future in the routine diagnostics sufficiently suitably.

Likewise subject-matter of the invention is containing a system therefore for the release and/or isolation of nucleic acids from a suspension of biological Kompartimenten, the components

- a unit 10 for the admission one or several sample working on containers (A),
- a unit 20 to the Thermostatisierung of the sample working on containers (A) and therein contained fluids,
- a unit 30 for vibrating the sample working on containers (A) and
- a unit 40 for the magnetic disconnection of the magnetic particle to a wall of each sample working on container (A),

in coupling co-ordinated one on the other.

The unit 10 has prefers the possibility of the admission of several sample working on containers. Particularly preferentially the possibility of the admission of micro titer plates exists 96 in the waving format. Preferred this system contains additionally a unit 50 to the distance of fluid from the sample working on container (A). Likewise preferentially the units are stored 40 and 10 relatively to each other movable. In addition preferentially the wise sample working on containers (A) a lower discharge opening (A11) up, which are connected with a suction device 50 or can be connected.

In Fig. 1 and 2 is schematically shown a system with units according to invention:

The module 10 takes up in or several sample working on containers (A) and ensures for the fact that the heat transfer is optimized according to the demanded heating and cooling rates. The module provides for a minimum deviation of the temperature from cavity to cavity. The module takes the temperature medium (z. B. Water) up and gives the warmth and/or. Cold weather purposefully toward sample working on container.

The module takes the mechanics 40 to the movement of the magnets on (magnets and rotary shafts). The engine can be outside of the module, z. B. on the framework positions.

The module connects the sample working on containers for common mixing. The module is connected with the mixing device 30.

The module takes up the suction hoses 51 for the suction from the sample containers to removing fluids (the Waste). The module is between sample working on container and a socket 13, z. B. from polysulphone, sealed, thus when sucking the Waste no air off between sample working on container and Inlet 14, block, z. B. from aluminum with holes 12, one sucks in.

The module has a surface which can be cleaned easily and protects the user against combustions (z. B. by a plastic coating).

Unit 20 essentially consists of liquid keeping at a moderate temperature elements, a 3/2-Wege valve, wires 21, heating, radiator and circulation pump. The keeping at a moderate temperature reservoir outside of the module is around a multiple more largely than the dead volume of the DNA module thereby after switching the 3/2-Wege-Ventils the variable disturbance is minimized. The heating and the radiator keep at a moderate temperature in the forward motion and are if necessary clocked. A regulation switches the valve, heating and cooling, together with which suitable flow rate of the circulation pump the desired heating and cooling rates become reached.

Alternatively the unit 20 consists of dry keeping at a moderate temperature elements. The heating staffs to heating and Peltiers for cooling are integrated directly in the DNA module. Advantage: No liquid flow system in this extreme temperature range.

The unit 30 mixes and resuspendiert. A stepping motor with an eccentric and a balance mass drive 11 put on complete DNA module from a solid framework, that over vibration dampers into a circular eccentric course solid amplitude and variable frequency. The amplitude is A @ 1.5 mm, the frequency 1 @ f @ 50 cycles per second. The mixing and/or. Resuspensionsdauer amounts to < depending upon physical properties of the sample material between 5; t < 30 S. By replacement of the Excenters it is possible in addition, in few minutes to vary the amplitude manual.

The unit 40 consists of a toothed belt, which changes the rotary motion of the DC engine on a side of the DNA module with in each case four shafts to the admission of 4 magnets each and toothed wheel at the faces into the circulation of the magnets. The two final positions are detected with in each case a light barrier. On the opposite side of the DNA module is exactly the same arrangement, to move so that the magnets of each side synchronously opposite one on the other. In this case twice as many magnets are used such as vessels.

In an alternative arrangement for n vessels n+1 magnet are used. Here the same magnet between two neighbouring vessels are led, one save thus n-1 [= 2n (n+1)] Magnets. In the position ?ON? has the magnet maximum effect on the Magnetbeads. In the position ?OFF? is so far from the vessel the magnet that he does not have effect on the Magnetbeads. The driving time between the end positions ?ON? and/or. ?OFF? amounts to t < 1.5 S.

The coupling of the components of the system is on the one hand functional, z. B. by integration of the magnets into the unit 10 to understand and on the other hand temporally z. B. by control of the enterprise of the units into for the desired use of suitable succession; this can for example taken place with a computer program or by means of Initiation of the individual Indexing steps with the user.

In Fig. a method according to invention is pointed 3 to the isolation of nucleic acids. On this figure with in the following the examples described description of a method cover is taken. The sample container is in an admission in unit 10, whereby preferentially at the sample container a bar A20 is intended, that the interior form of the admission is adapted (z. B. conical external form). The vessels shown in the profile can be made in a simple manner injection moulding-technically of polypropylenes.

A main advantage of the invention is that the system to far extent can be adapted on the use of different values by magnetic particles. It is relatively flexible and in most different methods applicable.

The subject-matter of the Invention is more near described by the following example.

Example 1

With the method according to Invention it concerns a method, whose admits fundamentals to the person skilled in the art from the nucleic acid diagnostics are. As far as experimental details are not implemented in the following, becomes to the full extent on Molecular Cloning, publisher J. Sambrook et al., CSH 1989 cover taken.

In a special embodiment of the method according to Invention for the working-up of nucleus-acid sample solutions, the following work procedures are accomplished (see Fig. 3). In a first step (I) a zellhaltige sample liquid in a sample container A with a material inkubiert, to which the cells are bound, from which nucleic acids will be won are. For this this material can exhibit either specific binding characteristics for the surface of the cells, z. B. by immobilization of antibodies against surface antigens or an absorber substance (A16, not shown), it can be intended however also a material with filter characteristics (A15, not shown), by which the cells are held back, if the fluid depresses through the material, z. B. from the sample container one removes. Conditions for the immobilization of cells at surfaces are well-known the person skilled in the art, z. B. from Methods in Enzymology of volume. 171, Biomembranes/part R transport Theory: Cell and Model Membranes, Edited by Sidney butcher, Becca butcher, department OF Molecular Biology, Vanderbilt University, Nashville, threshing floor lake, pages 444 FF or 581 FF.

During the Inkubation the sample container is preferentially by a cover B sealed, over active and/or. to ensure passive contamination protection.

In a further step the fluid is removed from the sample container, while cells, whose nucleic acids insulated stay to become to be supposed, in condition in the sample container, bound to the material. Since it concerns with the cellbinding material individual materials, a retaining can be achieved by the fact that the material is magnetic (manufacturers: Dynal, Oslo, Norway is advanced from the outside) and the magnet to the sample container. The fluid can be sucked off by the discharge opening A11 under creation of an easy vacuum. For this a valve is intended at the discharge opening, which by creation of underpressure opens.

To the large distance of possibly disturbing sample components of the cells one or more wash steps are planned. For this the sample container a washing liquid is filled in, in which possibly impurities separate, which impair however the connection of the cells to the surface of the cellbinding material not substantially. Such washing solutions are the person skilled in the art z. B. from cell separate ion minutes and/or. from appropriate cleaning kit minutes for nucleic acids admits. They essentially depend on the kind of the connection of the cells to the material.

After the last washing solution from the sample container A was sucked off if necessary, the cleaned, enriched cells are brought with a suitable Lyseflüssigkeit to the release of nucleic acids from the cells in contact. The reagents of these Lyselösung depend to a large extent on the kind of the immobilized cells (Rolf's et al.: PCR, Clinical DIAGNOSTICs and Research, Springer publishing house, 1992, S. 84 FF). If it concerns with the cells bacteria, contains the Lyselösung prefers Proteinase K to the dismantling of the cell wall. Wishing case becomes the Lyse by heating up and/or. Cooling as well as mixing of the reaction mixture by vibrating the sample container support. At the end of this explanation too insulating nucleic acids are present freely in the solution.

Also during the Lyse the reaction container is preferential by a cover sealed, in order to prevent contaminations from the environment. After end of the Lyse the cover, preferentially with the help of an appropriate mechanical apparatus, is removed. Afterwards into the sample container, which contains a mixture of dismantling products of the cells as well as nucleic acids, a moulded product C is inserted, whose outside outline C12 is co-ordinated with the internal outline A17 of the sample container. This moulded product is hollow and in the direction of the sample container and the relation mixture by a filter C11 (porous matrix) sealed. The introduction of the moulded product C effected prefers B11 of the cover B, which in addition a component contains B10 with the help of an element, which is suitable for the closure of the sample container. To this case the moulded product with the cover is introduced seized (II) and simultaneous with the closure of the sample container into the sample container. During this event in addition the reaction mixture will penetrate C11 by the filter into the cavity C14 of the moulded product (IV). On the one hand large particles can be prevented by planning the filter from the entrance into the cavity and on the other hand because of the nucleic acid-binding properties during the Durchtritts of the reaction mixture a connection of nucleic acids to the filter are already reached. In this case a glasfaserhaltiges filter material is selected.

In a next step the remaining Lysereaktionsmischung far away from the apparatus by sucking off by the discharge opening A11 in the sample container, formed by A and C. Also the solution penetrated into the hollow body C14 of the moulded product thus one removes, so that the filter contains if possible no more liquid remainders. Afterwards the

cover B used so far is removed, whereby the moulded product C remains first in the sample container (engaged) (V).

Simultaneous one or afterwards is prepared a Elutionsgefäss D for the admission of the moulded product C (either in the system according to invention or outside). If necessary on this vessel present covers one removes (VI). Preferred before transferring of the moulded product C into the Elutionsgefäss D a Elutionslösung is submitted to z into the Elutionsgefäss. B. in-pipetted. The composition of the Elutionslösung depends on the kind of the connection of nucleic acids to the material in the filter C. It contains reagents, under whose effect immobilized nucleic acids of the material eluiert, of D. h. solved, become. That the Elutionsgefäss locking covers B is originally attached to the sample container A with the moulded product C (VII).

To the removal of the moulded product C from the sample container A the moulded product C with the cover B is removed (VIII). The combination of cover and moulded product is introduced afterwards to the Elutionsgefäss (IX). Preferred contains moulded product C means (C13, not shown) to adjustment moulded product in Elutionsgefäss D, which cause that the moulded product only under destruction of the moulded product C or the vessel D or with a force, which is larger than the force, which is necessary for the solution of the cover B of the moulded product C, can be removed from the vessel D. A distance of the moulded product from the Elutionsgefäss is not intended.

During the penetration of the moulded product C into the Elutionsgefäss the submitted Elutionslösung penetrates into the filter C11 and replaces the immobilized nucleic acid of the solid matrix. Depending upon quantity of the submitted Elutionslösung either only the filter is soaked with the Elutionslösung or penetrates the Elutionslösung with again loosened nucleic acids into the hollow body C14. So that the Elution of nucleic acids runs as completely as possible, the internal contour of the Elutionsgefässes should be as closely as possible to the outer contour of the moulded product adapted.

In a following step the cover B of the combination of moulded products C and Elutionsgefäss D is removed (X). It is used, in order to take up (XI) and to the cavity of the moulded product C introduce a stamp E (XII). This cover reaches from the inside into the stamp E. The stamp becomes so strong against the filter C11 compressed that fluid from the filter penetrates by an opening in the pressure surface into an interior of the stamp. This event is particularly effective, if the pressure surface in its outside outline at least in the range, within which pressing out is to take place, to which internal outline of the moulded product C is adapted. The stamp E can preferentially in this layer, z. B. by engaging, to be fixed. Since in such a way formed apparatus is relatively well sealed by the cover, the nucleus-acid solution in the apparatus can be kept.

Inferred to the removal of a desired quantity of nucleic acid solution the cover knows far away (XIII) and over an opening of the interior of the stamp the desired quantity, z. B. in a pipetting procedure (XIV). Subsequently, the cover can be remounted.

In the following flow chart the fitting the described method is indicated.
EMI18.1

Manual work procedures are fat-printed represented. Non--manual work procedures or partial expirations are called by operation for example a key.

In the following the sample container A tube, Elutionsgefäss D as BACK-UP vessel, moulded product C as mat employment and stamp E are called as pressing out stamps.
EMI19.1

EMI20.1

EMI21.1

Wishing case the suction hoses and the recesses are rinsed by means of a Reinigungsflüssigkeit and cleaned thus (forwards and/or. after lead-through of the method and in absence of the sample containers. Reference symbol list A sample container 10 inlet port
11 discharge opening
17 internal form
19 external form
20 circulating bar
22 element for the adjustment of further functional elements B cover 10 device to the sealing of the sample container A
11 device for seizing the moulded product C C moulded product 11 porous matrix
outline expresses 12
13 compositions for the adjustment of the moulded product in the Elutionsgefäss
14 hollow bodies
15 agents to the fixing of a cover
16 internal outline
17 compositions for the adjustment of a stamp E, circulating
18 circulating bar, interruptibly
19 edge D Elutionsgefäss 12 engaging notch E stamp 10 pressure surface
11 outer contour
12 interior
13 openings in the pressure surface
14 withdrawal opening
15 seal
16 engaging ring
17 aperture apparatus 1 framework
10 unit for the admission of sample containers
11 oscillation steam
12 hole for the admission of A

13 sockets
14 Inlet for heating and cooling A
20 unit to the Thermostatisierung of sample containers
21 cooling/heating medium wire
30 unit for vibrating Probengefässen/Excentermotor
40 unit to the magnetic separation of magnetic particles
41 axles to the twisting of the magnet segments
42 magnet segments
50 vaccum pump
51 (negative pressure) tube